



Florida Department of Health



World Health Organization

WHO post-outbreak biosafety guidelines for handling of SARS-CoV specimens and cultures

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During the SARS epidemic between November 2002 and June 2003, a large number of specimens were collected from suspected and confirmed SARS human cases and sent to different national and international laboratories for a variety of pathological tests. WHO's guidelines for the safe handling of those specimens during the outbreak period were described in [WHO biosafety guidelines for handling of SARS specimens](#). In addition, many specimens have been obtained from animal sources during investigations into the origin of SARS-CoV, and these should also be subject to the same guidelines for safe handling of specimens.

The following WHO biosafety guidelines have been prepared for handling SARS-CoV specimens in the post-outbreak period. The guidelines take into account the absence of chains of human transmission, and highlight the importance of strict adherence to biosafety procedures and practices for laboratory work with SARS-CoV. As detailed below and in previous biosafety guidelines, WHO strongly recommends Biosafety Level 3 (BSL3) as the appropriate containment level for working with live SARS-CoV material.

The possibility that a SARS outbreak could occur following a laboratory accident is a risk of considerable importance, given the relatively large number of laboratories currently conducting research using the SARS-CoV or retaining specimens from SARS patients. These laboratories currently represent the greatest threat for renewed SARS-CoV transmission through accidental exposure associated with breaches in laboratory biosafety.

Given the severity of the threat, WHO strongly recommends that national governments maintain a registry of laboratories that are approved to safely and securely hold and work with specimens of suspected or confirmed SARS patients or cultures containing SARS-CoV.

Appropriate national authorities should provide guidelines for laboratories to catalogue and control the storage of cultures and specimens of SARS-CoV for periodic inspections.

WHO also encourages the destruction of unwanted or unneeded clinical and animal specimens suspected or confirmed of containing SARS-CoV, and/or of stocks of SARS-CoV, that cannot be kept under secure conditions.

Any laboratory accidents, e.g. accidental spillage of material suspected of containing SARS-CoV should be reported to the appropriate authority, and all people potentially exposed to SARS-CoV resulting from such accidents should be closely followed for a period of 10 days for evidence of SARS-CoV infection. In addition, any cluster of acute lower respiratory tract illness in such a laboratory should be rapidly investigated to exclude SARS-CoV infection. Depending on the nature and severity of the illness, the exclusion of SARS-CoV infection may also be necessary in the event of sporadic cases of acute respiratory illness which do not fulfil the current [WHO clinical case definition for a SARS alert](#).

To ensure the safety of laboratory personnel and to mitigate the potential for laboratory accidents in the post-outbreak period, WHO recommends the following guidelines for staff handling SARS-CoV, or specimens which may possibly contain SARS-CoV:

WHO biosafety guidelines for handling SARS-CoV specimens and viral stocks.

In cases where laboratory facilities do not meet at least basic laboratories – Biosafety Level 2 (BSL2) containment conditions, consideration should be given to referral of specimens to suitably equipped reference laboratories for primary diagnostic tests.

The following activities may be performed in **BSL2 facilities** with appropriate basic laboratories – **Biosafety Level 2 (BSL2)** work practices, as described in the [WHO Laboratory Biosafety Manual, 2nd revised edition](#).

- Routine diagnostic testing of serum and blood samples (including haematology and clinical chemistry)
- Manipulations involving neutralized or inactivated (lysed, fixed or otherwise treated) virus particles and/or incomplete, non-infectious portions of the viral genome
- Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

The following activities may be performed in **BSL2 facilities with additional BSL3 work practices**:

Examples of activities that require BSL3 working practices for work with SARS-CoV in BSL2 facilities include:

- Aliquoting and/or diluting specimens
- Inoculation of bacterial or mycological culture media
- Performance of diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
- Nucleic acid extraction procedures involving untreated specimens
- Preparation and chemical- or heat-fixing of smears for microscopic analysis

BSL3 practices include:

- Any procedure that may generate aerosols or droplets should be performed in a biological safety cabinet (e.g., sonication, vortexing).
- Laboratory workers should wear protective equipment, including disposable gloves, solid-front or wrap-around gowns, scrub suits, or coveralls with sleeves that fully cover the forearms, head covering and, where appropriate, shoe covers or dedicated shoes, eye protection and a surgical mask, or full-face shield, because of the risk of creating aerosols or droplets exposure when performing specific manipulations.
- Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be unloaded in a biological safety cabinet.
- Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against enveloped viruses should be sufficient if used according to the manufacturer's recommendations. Generally, 5% bleach solutions are appropriate for dealing with biohazardous spillage. More information on disinfection and sterilization is provided in the [WHO Laboratory Biosafety Manual, 2nd revised edition](#).
- Biological waste contaminated with suspect or confirmed SARS specimens, or with SARS-CoV, should be treated as outlined in the [WHO Laboratory Biosafety Manual, 2nd revised edition before disposal](#).

WHO strongly recommends that the BSL3 precautions described above are adopted and followed for work in BSL2 laboratories with SARS specimens.

When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g. respirators, face shields) and physical containment devices (e.g. centrifuge safety cups or sealed rotors) must be used.

The following activities should be performed in containment laboratories - **Biosafety Level 3 (BSL3)**, by personnel trained in the use of appropriate **BSL3 work practices**.

- Performance of diagnostic tests that involve propagation of viral agents in vitro or in vivo
- Work involving the replication of SARS-CoV in cell culture and/or storage of cell culture isolates
- Recovery of viral agents from cultures of SARS-CoV specimens
- Manipulations involving growth or concentration of SARS-CoV

The following activities require **Animal BSL3 facilities and Animal BSL3 work practices**:

- Animal studies with live SARS-CoV or with closely related viruses from wildlife sources
- Any protocol involving animal inoculation for confirmation and/or characterization of putative SARS agents

Recommendations of the WHO SARS Laboratory Workshop, Geneva, 22 October 2003

An informal SARS Laboratory Workshop was held at WHO, Geneva, on 22 October 2003, to discuss aspects of the laboratory diagnosis of SARS-CoV infection pertaining to the standardization of test protocols and reagents, the development of a panel of positive control sera for diagnostic serology, the development of a set of strategic plans or algorithms to provide guidance to laboratories about testing of specimens from patients with atypical pneumonia in non-epidemic periods, and biosafety and biocontainment issues in laboratories working with live SARS-CoV. A summary of the discussion and recommendations of the meeting is available at [Summary of the discussion and recommendations of the SARS Laboratory Workshop, 22 October 2003](#) .

Transport of specimens

Transport of specimens within national borders should comply with current national regulations.

International air transport of human specimens from suspect or probable SARS cases, or of specimens from SARS-CoV infected animals must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations.

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- Consignment of diagnostic specimens 2003

IATA regulations of 2003 allow specimens known or suspected of containing the SARS agent to be transported as UN 3373 “Diagnostic Specimens” when they are transported for diagnostic or investigational purposes.

Specimens transported for any other purposes, and cultures (as defined in the IATA regulations) prepared for the deliberate generation of pathogens, must be transported as UN 2814 or UN 2900, as appropriate.

All specimens to be transported (UN 3373, UN 2900, or UN 2814) must be packaged in triple packaging consisting of three packaging layers:

UN 3373, Diagnostic Specimens, shall be packed in good quality packaging, which shall be strong enough to withstand the shocks and loads normally encountered during transport. Packaging shall be constructed and closed

so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration or by changes in temperature, humidity or pressure.

Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging shall be placed in a final outer package with suitable cushioning material. Any leakage of the contents shall not substantially impair the protective properties of the cushioning material or of the outer packaging.

For Liquids

The primary receptacle(s) shall be leakproof and shall not contain more than 500 ml. There shall be absorbent material placed between the primary receptacle and the secondary packaging; if several fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them. The absorbent material shall be in sufficient quantity to absorb the entire contents of the primary receptacles and there shall be a secondary packaging that shall be leakproof. The primary receptacle or the secondary packaging shall be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar). The outer packaging shall not contain more than 4 litres.

For Solids

The primary receptacle(s) shall be sift proof and shall not contain more than 500 g. If several fragile primary receptacles are placed in a single secondary packaging, they shall be either individually wrapped or separated so as to prevent contact between them and there shall be a secondary packaging which shall be leakproof. The outer packaging shall not contain more than 4 kg.

For air transport, the smallest overall external dimension of a completed package must be at least 10 cm.

Packaging must conform to certain performance standards.

For further information about definitions, packaging requirements, markings and labels, accompanying documentation, and refrigerants, please refer to the competent authority, current IATA shipping guidelines, commercial packaging suppliers, or available courier companies.